Journal of Forensic and Legal Medicine 23 (2014) 26–31

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Case report

Hair analysis to demonstrate administration of amitriptyline, temazepam, tramadol and dihydrocodeine to a child in a case of kidnap and false imprisonment

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ARTICLE INFO

Article history:
Received 8 July 2013
Received in revised form
5 December 2013
Accepted 19 January 2014
Available online 25 January 2014

Keywords:
Children's hair
Administration
Amitriptyline
Temazepam
Tramadol
Dihydrocodeine

ABSTRACT

Amitriptyline, temazepam, tramadol and dihydrocodeine are prescription-only-medications that are rarely prescribed to children. Each of these drugs has a sedative effect on the central nervous system; their combined use could cause an exacerbation of the sedative effects. Amitriptyline (a tricyclic antidepressant) can be prescribed to treat nocturnal enuresis; temazepam (a hypnotic) can be used as a premedicant in inpatient and day-case surgery; tramadol (a synthetic opioid analgesic) is used to treat moderate or severe pain, though it is not recommended for children under the age of 12 years and dihydrocodeine (opioid analgesic), which is available in combination with acetaminophen (Co-dydramol®), is not recommended for children under the age of 4 years; in children over 4 years, a reduced dose is necessary. The North West Forensic Science Service Laboratory, Euxton, Lancashire, was asked by a British police force to analyze three separate hair samples, which had been collected from a young child following their discovery as a result of a large scale kidnap and false imprisonment investigation. After decontamination and segmentation (20 x 1-cm section), two of the three hair specimens were analyzed by liquid chromatography coupled with tandem mass spectrometry after alkaline (pH 9.5) extraction using methylene chloride/isopropanol/*n*-heptane (25:10:65, v/v/v). The entire length of each hair specimen tested positive for amitriptyline and nortriptyline (7–314 pg/mg amitriptyline; 7–318 pg/mg nortriptyline), temazepam (2–29 pg/mg), tramadol (60–2000 pg/mg) and dihydrocodeine (10–90 pg/mg) demonstrating that the child had ingested these drugs on more than one occasion prior to the kidnap. In this case, the child's mother and the mothers' partner were found guilty of kidnap, false imprisonment and perverting the course of justice. There are very few studies citing the concentrations of these drugs in children – especially children's hair samples. This case demonstrates the added value of hair testing and emphasizes the importance of using hair samples to complement conventional analysis.

1. Introduction

There is limited data in the literature regarding drug concentrations of amitriptyline, nortriptyline, temazepam, tramadol and dihydrocodeine in children's hair specimens as these drugs are rarely, or never, prescribed to young children. Blood and urine remain the matrix of choice for toxicology analysis and investigation into ingestion (accidental poisoning/neglect) or administration (murder/manslaughter). In cases of suspected administration, the hair matrix has the advantage of being able to confirm exposure over an extended time period – far beyond the capabilities of the more conventional samples – due to the larger detection window. In addition, segmental hair analysis can identify multiple exposures and, in some instances, identify patterns of drug use/administration. The lack of published data on hair concentrations in children makes interpretation of analytical results problematic; one cannot simply apply the same interpretation (for adults) to children's hair testing results. The growth of children's hair is more variable[1,2] than adults and significantly less data is available concerning controlled laboratory studies on drug intake and subsequent pharmacological effects.

Amitriptyline (Elavil®) belongs to the tricyclic group of antidepressants and is a prescription-only drug that is used mainly during

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http://dx.doi.org/10.1016/j.jflm.2014.01.005

the long-term treatment of depression in adults; the drug is also prescribed in adults to treat neuropathic pain and migraine. Amitriptyline can be prescribed to children to treat nocturnal enuresis, i.e., involuntary passing of urine (bed-wetting). More sedating than similar drugs, amitriptyline is useful when depression is accompanied by anxiety or insomnia. Taken at night, the drug encourages sleep and helps to eliminate the need for additional sleeping drugs. Side effects associated with the use of amitriptyline include, amongst others, sedation, nausea, sweating and headache. If taken in excess, amitriptyline may cause coma, seizures and abnormal heart rhythms.[3–5]

Temazepam belongs to the benzodiazepine group of drugs and is a prescription-only hypnotic, i.e., sleep-inducing drug that is used in the short-term treatment of insomnia. Benzodiazepines are generally prescribed for the short-term relief of severe anxiety; long-term use should be avoided. These drugs can be effective in alleviating anxiety states, however they should only be prescribed to children for the relief of acute anxiety (and related insomnia) caused by fear, e.g., before surgery, as amnesia reduces the likelihood of any unpleasant memories of the procedure. Temazepam acts for a shorter period of time than some other benzodiazepines, e.g., diazepam (Valium®) and nitrazepam (Mogadon®) and induces little, if any hangover effects. Withdrawal phenomena are more commonly experienced with short-acting benzodiazepines. Effects typically associated with the use of temazepam include dizziness, confusion, drowsiness, reduced alertness, slowed reactions, impaired ability to concentrate and impaired co-ordination.[3–5]

Tramadol (Zydol®) is a synthetic opioid analgesic that is chemically similar to natural opioids. It is a prescription-only drug that is used to prevent or treat moderate pain and severe pain associated with, for example, a heart attack, injury, surgery, or cancer. This drug is not recommended for children under the age of 12 years. Side effects associated with the use of tramadol include nausea and vomiting (particularly in the initial stages of use), hallucinations, dizziness, confusion, drowsiness, headache and fatigue.[3–5]

Dihydrocodeine is a prescription-only opioid analgesic that is related to codeine (and morphine) and is of similar potency to codeine. The drug is primarily used for the relief of mild to moderate pain though it has also been used as a cough suppressant. Dihydrocodeine is available in combination with acetaminophen (Co-dydramol®); in this way a lower dose of the opioid can be used to give pain relief with fewer side effects. This drug is not recommended for children under the age of 4 years; in children over 4 years, a reduced dose is necessary. Side effects associated with the use of dihydrocodeine are similar to those of tramadol; common side effects include nausea, vomiting, drowsiness, dizziness and headache.[3–5]

In recent years, considerable information about drug-facilitated crimes (rape, sexual assault, robbery, sedation of children) has accumulated.[6–10] In these situations, the victims are subjected to non-consensual acts while they are incapacitated through the effects of a drug. This impairs their ability to resist or to give consent to the act. In a typical scenario, a predator (rapist, robber) surreptitiously spikes a drink of an unsuspecting person with a hypnotic drug. Victims usually report loss of memory during and after the event. For the perpetrator, the ideal substance is one that is readily available, is easy to administer, rapidly impairs consciousness and causes anterograde amnesia (i.e., it prevents the recall of events that occurred whilst under the influence of the drug but not general memory).

Blood and urine are the conventional specimens to document drug exposure. However, in cases of drug-facilitated crime, hair samples are necessary to complement these analyses as hair can permit differentiation of a single exposure from chronic use of a drug by segmentation of the hair for a stated growth period.[11–13] Moreover, due to a frequent long delay between the incident and police declaration, hair can be the only solution for a lack of corroborative evidence of a committed crime.

Beside the classic sexual assault and robbery cases, there has been an increase in the number of cases where a drug was administered to induce sedation.

This paper will focus on the difficulties and the specificities of drug exposure in a dramatic case dealing with a child.

2. Case history

In February 2008, a British police force received a telephone call from the mother of a 9 year old child, informing them that the child had not returned home from school. Extensive searching and enquiries resulted in the child being located twenty-four days later, at the home address of a friend of the child's mother. It was suspected that the child may have been administered drugs during the time period between disappearance in February and discovery in March. Numerous medications were recovered and seized during searches of both the mothers' home address and the address where the child was discovered. Investigations revealed that the following medications were prescribed to the friend of the child's mother: amitriptyline hydrochloride; clonazepam; dihydrocodeine; temazepam; tramadol. Meclozine (TRAVELeeze®) – an antihistamine with antiemetic action to prevent nausea and vomiting, particularly travel sickness, was also found at this address.

Initial analysis of a urine sample that was collected from the child shortly after their discovery, identified meclozine and a trace amount of temazepam (less than 10 μg/L). This demonstrated the recent ingestion of these drugs, i.e., ingestion within 48–72 h of sample collection, and prompted the forensic toxicologist to request that hair samples be collected from the child as the urine result, in isolation, would offer little assistance in determining whether the child had ingested drugs at an earlier time during the period of captivity. The extended window of detection provided by the hair matrix could investigate this issue analytically and provide added value to the police investigation.

The British police force collected three separate hair samples: two samples were collected in April 2008 and one sample was collected in May 2008. The locks of hair were greater than 12, 15

Table 1
Results of segmental drug analysis for the presence of amitriptyline and nortriptyline. All concentrations are in picograms per milligram (pg/mg).

Section	Amitriptyline	Nortriptyline	Amitriptyline	Nortriptyline
	Sample collected in April 2008		Sample collected in May 2008	
0–1 cm	13	13	8	10
1–2 cm	7	7	7	8
2–3 cm	12	11	9	9
3–4 cm	25	21	13	13
4–5 cm	49	38	28	27
5–6 cm	71	61	50	55
6–7 cm	93	81	51	71
7–8 cm	89	79	66	71
8–9 cm	93	84	71	94
9–10 cm	187	168	91	128
10–11 cm	294	251	105	136
11–12 cm	206	174	145	219
12–13 cm	199	168	117	155
13–14 cm	179	175	141	182
14–15 cm	234	197	144	187
15–16 cm	253	217	260	273
16–17 cm	264	212	182	209
17–18 cm	254	182	236	195
18–19 cm	214	186	196	226
19–20 cm	255	216	146	168
end	314	318	75	88

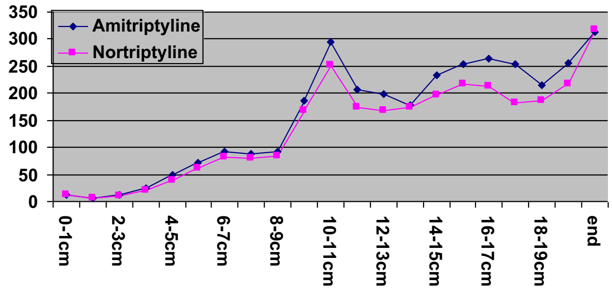

Fig. 1. Results of segmental analysis for the presence of amitriptyline and nortriptyline in hair sample collected April 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

and 18 cm in length respectively and were brown in color. The orientation of the hair samples had been maintained. It was requested that the hair samples be analyzed for a range of sedative and pharmaceutical/medicinal drugs by segmentation. The considerable difference in the length of the hair samples suggests that the hair was collected from different sites on the child's head.

3. Materials and methods

3.1. Chemicals and reagents

Acetonitrile, methylene chloride, isopropanol and *n*-heptane were HPLC-grade (Merck, Darmstadt, Germany). Chemicals for the ammonium formate buffer used in the mobile phase, ammonium chloride buffer used for the extraction [$(NH_4)_2Cl$, adjusted to pH 9.5], and formic acid were purchased from Fluka (Saint-Quentin Fallavier, France). Diazepam-d_5 and tramadol-d_3 were purchased from Promochem (Molsheim, France).

3.2. Extraction

The hair strands were washed/decontaminated twice using methylene chloride (5 mL, 2 min) and then segmented (segments of 1 cm). Each segment was cut into small pieces (<1 mm) and approximately 50 mg of the hair segments were incubated overnight (without agitation) at 40 °C in 1 mL of ammonium chloride buffer at pH 9.5, in the presence of internal standard, diazepam-d_5 (10 ng/mg) and/or tramadol-d_3 (50 ng/mg).

After a liquid–liquid extraction with 5 mL of a mixture of methylene chloride/isopropanol/*n*-heptane (25:10:65, v/v/v) and evaporation to dryness, the residue was reconstituted in mobile phase (acetonitrile/2 mM formate buffer – 20:80, v/v).

Table 2
Results of segmental drug analysis for the presence of temazepam. All concentrations are in picograms per milligram (pg/mg).

Section	Temazepam Sample collected in April 2008	Temazepam Sample collected in May 2008
0–1 cm	5	3
1–2 cm	N/D	3
2–3 cm	3	4
3–4 cm	6	6
4–5 cm	4	6
5–6 cm	2	10
6–7 cm	7	11
7–8 cm	6	9
8–9 cm	7	8
9–10 cm	16	13
10–11 cm	24	8
11–12 cm	14	12
12–13 cm	16	16
13–14 cm	16	21
14–15 cm	18	17
15–16 cm	22	18
16–17 cm	27	16
17–18 cm	20	18
18–19 cm	21	18
19–20 cm	27	15
end	29	N/D

3.3. LC-MS/MS procedure

Analysis was performed using a Waters Alliance 2695 system (North Brunswick, NJ) in the electrospray positive mode. Chromatography was achieved using an XTerra® MS C_{18} column (100 × 2.1 mm, 3.5 µm) eluted at a flow rate of 0.2 mL/min with a gradient of 5/95% (tramadol) or 20/80% (amitriptyline). The mobile phase was acetonitrile and ammonium formate buffer (5/95% v/v). Injection column was 10 µL (tramadol) or 20 µL (amitriptyline/nortriptyline). A Quattro Micro triple-quadropole MS (Micromass-Waters, Milford, MA) fitted with a Z-spray ion interface was used for analyses.

3.4. Method validation

Criteria for samples to be considered positive include the following: Retention time ±0.2 min of the standard (tramadol); presence of two transitions (with a signal to noise ration greater than 3); correct ratio between the two transitions (compared to the standard).

Under the chromatographic conditions used, there was no interference with the analytes by chemicals or any extractable endogenous materials present in hair. The calibrators were matrix

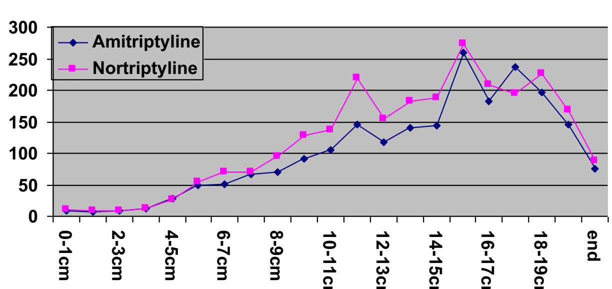

Fig. 2. Results of segmental analysis for the presence of amitriptyline and nortriptyline in hair sample collected May 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

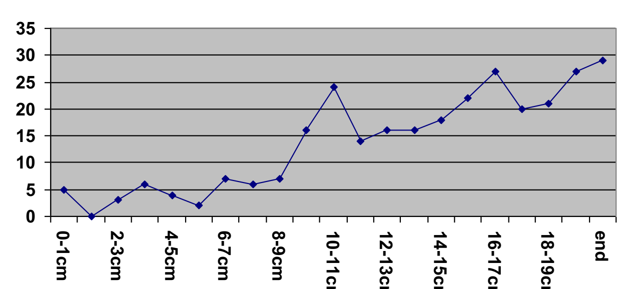

Fig. 3. Results of segmental analysis for the presence of temazepam in hair sample collected April 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

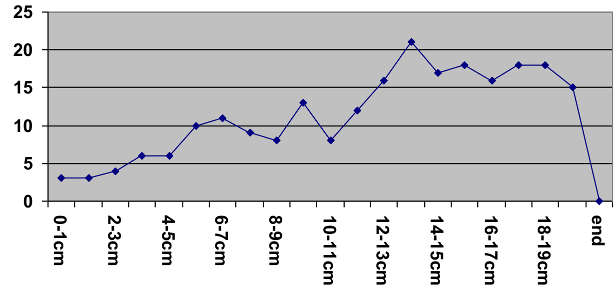

Fig. 4. Results of segmental analysis for the presence of temazepam in hair sample collected May 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

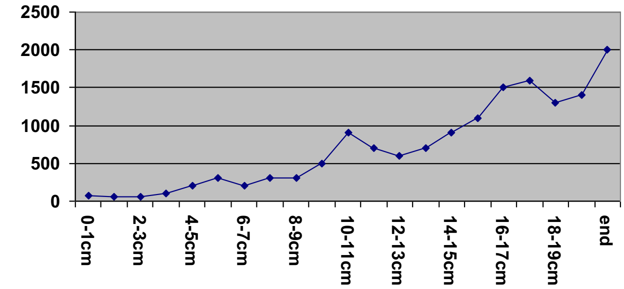

Fig. 5. Results of segmental analysis for the presence of tramadol in hair sample collected April 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

matched and checked prior to each new batch. Spiked hair samples were used for validation.

Linearity was observed for concentrations ranging from 0.1 to 50 ng/mg (tramadol, dihydrocodeine) and for concentrations ranging from 0.05 to 10 ng/mg (amitriptyline/nortriptyline and temazepam) with a correlation coefficient of greater than 0.99 in all cases.

The LOQ was determined as the lowest concentration of linearity repeated 10 times, where the CV was less than 20%, with a signal to noise ratio greater or equal to 10. The limit of detection (LOD) was calculated starting from the LOQ to obtain a signal to noise ratio greater than or equal to 3.

The LOQ for tramadol and dihydrocodeine was 0.1 ng/mg, with an LOD of 0.03 ng/mg. The LOQ for amitriptyline and nortriptyline was 0.05 ng/mg, with an LOD of 0.017 ng/mg. The LOQ for temazepam was 0.01 ng/mg, with an LOD of 0.005 ng/mg.

The analytical methodology was performed as previously reported.[14–16]

4. Results and discussion

The results of the segmental analysis for amitriptyline/nortriptyline are presented in Table 1 and Figs. 1 and 2; the results of the segmental analysis for temazepam are presented in Table 2 and

Table 3
Results of segmental drug analysis for the presence of tramadol. All concentrations are in picograms per milligram (pg/mg).

Section	Tramadol	Tramadol
	Sample collected in April 2008	Sample collected in May 2008
0–1 cm	70	40
1–2 cm	60	30
2–3 cm	60	40
3–4 cm	100	60
4–5 cm	200	100
5–6 cm	300	200
6–7 cm	200	300
7–8 cm	300	200
8–9 cm	300	300
9–10 cm	500	400
10–11 cm	900	300
11–12 cm	700	500
12–13 cm	600	400
13–14 cm	700	600
14–15 cm	900	600
15–16 cm	1100	800
16–17 cm	1500	600
17–18 cm	1600	800
18–19 cm	1300	800
19–20 cm	1400	800
end	2000	700

Figs. 3 and 4; the results of the segmental analysis for tramadol are presented in Tables 3 and Figs. 5 and 6 and the results of the segmental analysis for dihydrocodeine are presented in Table 4 and Figs. 7 and 8.

The major practical advantage of hair testing compared to urine (or blood) testing for drugs is that it has a larger surveillance window – weeks to months (as opposed to a few days), depending on the length of the hair shaft. The different matrices, and tests therein, compliment each other; urine and blood analysis provides short-term information of exposure, whereas hair analysis provides historic or long-term information regarding exposure. Segmental analysis can provide additional information regarding single or repeat/long-term exposure.

On average, hair grows (in adults) at a rate of 1 cm per month, however there are reports of this rate varying between 0.7 and 2.2 cm per month.[17] In this case, the growth of the child's hair was investigated by monitoring it over a period of a few months; it was found, on average, to be approximately 1 cm/month (0.9 cm/month).

The section of hair tested in this case would therefore represent the exposure of the child for a period of approximately 20 months. The presence of amitriptyline, temazepam, tramadol and dihydrocodeine in the hair samples demonstrates the ingestion of these drugs (knowingly or otherwise) by the child in the months leading up to discovery in March 2008. The distribution of the drugs in the child's hair is worthy of further discussion.

If a drug is ingested, in similar quantities on each occasion, then one would expect to see a steady decrease in drug concentration moving along the shaft as a result of losses through washing etc. If ingested sporadically in varying quantities, then it is possible to observe similar concentrations of a drug in each segment along the hair shaft. Similarly, if the quantity of drug that was ingested steadily reduced, it is possible to observe similar concentrations of the drug in each segment along the hair shaft. In this particular case, the results were unusual as the respective drug

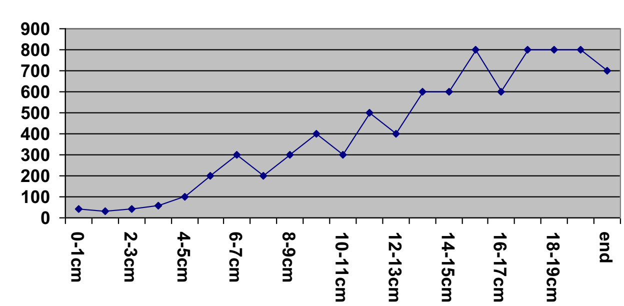

Fig. 6. Results of segmental analysis for the presence of tramadol in hair sample collected May 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

Table 4
Results of segmental drug analysis for the presence of dihydrocodeine. All concentrations are in picograms per milligram (pg/mg).

Section	Dihydrocodeine Sample collected in April 2008	Dihydrocodeine Sample collected in May 2008
0–1 cm	10	10
1–2 cm	10	10
2–3 cm	10	10
3–4 cm	10	10
4–5 cm	10	20
5–6 cm	20	40
6–7 cm	10	40
7–8 cm	20	30
8–9 cm	20	40
9–10 cm	40	50
10–11 cm	50	40
11–12 cm	40	60
12–13 cm	30	60
13–14 cm	40	80
14–15 cm	50	70
15–16 cm	50	90
16–17 cm	60	70
17–18 cm	60	80
18–19 cm	60	90
19–20 cm	60	70
end	90	60

concentrations increased moving along the hair shaft, away from the scalp. This suggests dosing on more than one occasion prior to the time the child went missing.

The presence of drugs in each hair segment does not necessarily mean that they were ingested during each representative time period. There is the possibility that the area of hair containing the drugs became more diffuse due to regular washing. Furthermore, drugs can be incorporated into the hair via sweat and variations in hair growth must be considered, i.e., on the scalp of an adult, approximately 85% of the hair is in the growing phase and the remainder is in the resting (or dormant) phase. Hair does not grow continually, but in cycles, alternating between periods of growth and periods of rest. There are three phases: the *anagen* phase where the follicle is actively producing hair; the *catagen* phase where the follicle enters a transition period during which time cell division stops and the follicle begins to degenerate and; the *telogen* phase where the hair shaft stops growing completely and the hair begins to shut down.

Based on the hair analysis results, it is likely that the child ingested the drugs on a number of occasions over an extended time period, possibly dating back more than 12 months prior to sample collection. The findings are not consistent with a single exposure/ingestion of these drugs. The increased concentration of the respective drugs in the 10–11 cm and the 16–17 cm segments of

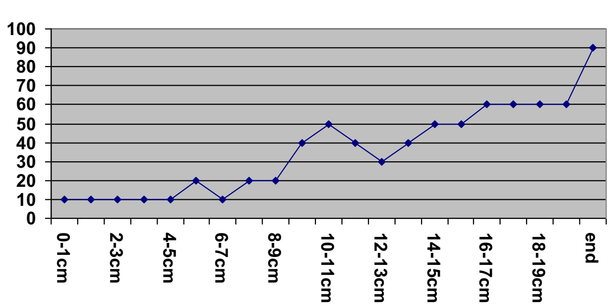

Fig. 7. Results of segmental analysis for the presence of dihydrocodeine in hair sample collected April 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

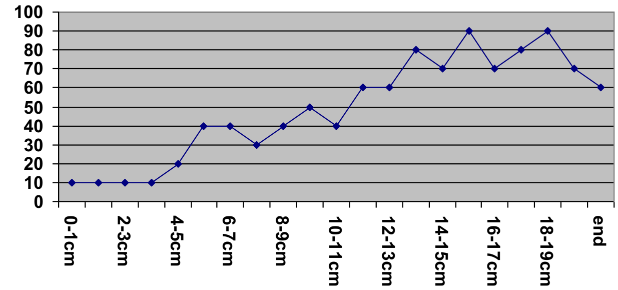

Fig. 8. Results of segmental analysis for the presence of dihydrocodeine in hair sample collected May 2008. All concentrations (*y*-axis) are in picograms per milligram (pg/mg).

hair offer support for the view that there was an increase in the amount of drug ingested at this time, i.e., an increase in the dosage and/or frequency of ingestion over this period. Accepting the variations in hair growth rate, these apparent 'peaks' in drug concentration could coincide with school vacation periods, i.e., Easter and summer breaks.

Urine analysis demonstrated drug (temazepam and meclozine) ingestion in the day or so prior to sample provision.

5. Conclusion

This case demonstrates the added value of hair testing and emphasizes the importance of using hair testing to complement conventional analysis. The hair matrix provides an increased window of detection and permits differentiation, by segmentation, of long-term use from a single exposure. Without hair analysis, this case would not have been fully investigated and evidence of amitriptyline, tramadol and dihydrocodeine administration to the child would have gone un-noticed.

The interpretation of drug concentrations in children's hair remains problematical; more data and published case reports are required, especially where information relating to admitted use (or administration) is known.

Ethical approval

None.

Funding

None.

Conflict of interest

None declared.

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